Hepatocellular carcinoma in patients with metabolic dysfunction-associated fatty liver disease: Can we stratify at-risk populations?

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Abstract

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a new nomenclature recently proposed by a panel of international experts so that the entity is defined based on positive criteria and linked to pathogenesis, replacing the traditional non-alcoholic fatty liver disease (NAFLD), a definition based on exclusion criteria. NAFLD/MAFLD is currently the most common form of chronic liver disease worldwide and is a growing risk factor for development of hepato-cellular carcinoma (HCC). It is estimated than 25% of the global population have NAFLD and is projected to increase in the next years. Major Scientific Societies agree that surveillance for HCC should be indicated in patients with NAFLD/MAFLD and cirrhosis but differ in non-cirrhotic patients (including those with advanced fibrosis). Several studies have shown that the annual incidence rate of HCC in NAFLD-cirrhosis is greater than 1%, thus surveillance for HCC is cost-effective. Risk factors that increase HCC incidence in these patients are male gender, older age, presence of diabetes and any degree of alcohol consumption. In non-cirrhotic patients, the incidence of HCC is much lower and variable, being a great challenge to stratify the risk of HCC in this group. Furthermore, large epidemiological studies based on the general population have shown that diabetes and obesity significantly increase risk of HCC. Some genetic variants may also play a role modifying the HCC occurrence among patients with NAFLD. The purpose of this review is to discuss the epidemiology, clinical and genetic risk factors that may influence the risk of HCC in NAFLD/MAFLD patients and propose screening strategy to translate into better patient care.

Key Words: Hepatocellular carcinoma; Metabolic dysfunction-associated fatty liver

disease; Nonalcoholic fatty liver disease; Surveillance for hepatocellular carcinoma; Incidence of hepatocellular carcinoma; Nonalcoholic steatohepatitis

Core Tip: Metabolic dysfunction-associated fatty liver disease (MAFLD) affects 25% of general population worldwide. Within that huge number of patients, a minority will progress to cirrhosis, with an annual incidence rate of hepatocellular carcinoma (HCC) > 1%. In them, surveillance for HCC by means of ultrasound with or without alpha-fetoprotein measurement is cost-effective. In patients with MAFLD cirrhosis who are men, older and diabetic, risk is even higher and magnetic resonance imaging might be a better screening test. However, the great challenge is stratifying the HCC risk in patients with MAFLD without cirrhosis. Factors that can help to stratify their risk (genetic, demographic, metabolic, non-invasive fibrosis tests) will be reviewed.

INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) previously known as non-alcoholic fatty liver disease (NAFLD) represents a condition of excessive accumulation of fat in the liver of people with features of metabolic syndrome regardless of alcohol consumption. Recently, a panel of international experts from 22 countries proposed this new nomenclature assigning the disease a name linked with its pathogenesis to overcome the negative definition originally attributed to NAFLD[1,2]. Definition of NAFLD was based on the presence of steatosis in > 5% of hepatocytes and the exclusion of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long-term use of steatogenic medications, and other known causes of liver disease[3]. On the contrary, definition of MAFLD is based on positive criteria, independently of the presence of other liver diseases. The diagnosis of MAFLD is based on the evidence of liver steatosis, in addition to one of the following three criteria: Overweight/obesity, presence of type 2 diabetes mellitus, or evidence of metabolic dysregulation[1,2]. Fat accumulation in the liver may be shown by histology, imaging (ultrasonography, controlled attenuation parameter by FibroScan®, magnetic resonance imaging–derived proton density fat fraction, computed tomography) or blood biomarkers (fatty liver index).

The Consensus panel further recognized that the exclusion of alcohol intake or hepatitis B virus (HBV) or hepatitis C virus (HCV) infections is no longer a prerequisite for the diagnosis of MAFLD. Patients who meet the diagnostic criteria for MAFLD and have in addition one of these concomitant diseases should be defined as having a dual etiology fatty liver disease[1]. Subsequently, expert panels of the Latin American Association for the Study of the Liver[4] and also from Middle East and north Africa[5] reached consensus to endorse the proposal on the redefinition of fatty liver disease and the new nomenclature (MAFLD).

MAFLD encompass a spectrum of conditions that may be limited to excessive liver fat (simple steatosis) or progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to hepatocellular carcinoma (HCC)[3]. MAFLD is mainly driven by hyperalimentation, unhealthy diets, sedentary behavior, leading to central and visceral adiposity, insulin resistance, overweight/obesity and metabolic syndrome. A recent meta-analysis, based on 86 studies from 22 countries estimated that global prevalence of NAFLD is 25.24% (95%CI: 22.10-28.65), peaking at 31.79%% in the Middle East, 30.45% in South America and as low as 13.48% in Africa[6]. The prevalence of NASH in the general population is not well known, since a liver biopsy is required to confirm this condition. It has been estimated that it can range from 1.5% to 6.45%[6]. One of the most worrying aspects of NASH is that modeling-projected prevalence (estimated in eight countries) is to increase by 15%-56% between 2016 and 2030[7]. Other Markov model-based study, but limited to United States, concluded that prevalent NAFLD and NASH cases are forecasted to increase 21% and 63%, respectively between 2015 and 2030, while incidence of decompensated cirrhosis and HCC will increase 168% and 137%, respectively, by 2030[8]. Results of both modeling studies suggest increasing cases of advanced liver disease in the coming years; taken together with an unmet of effective therapeutic approach means that MAFLD will be the leading cause of cirrhosis, liver transplantation and HCC in the next decades[7,8].

Primary liver cancer (PLC) is the sixth more frequent cancer in general population, with more than 900.000 new cases every year worldwide[9], but it ranks as the second one among cancer deaths, because of its bad prognosis[9,10]. Overall survival of patients is very low and the incident cases/deaths ratio is 0.9. HCC accounts more than 90% of all PLCs. The poor prognosis of HCC is mostly due to the fact that it usually emerges in patients with chronic liver disease and advanced fibrosis or cirrhosis. When patients are diagnosed by symptoms, they usually have large or multiple tumors. Then, the impaired liver function reserve due to cirrhosis prevents curative treatment by surgical resection; or the tumor extension is beyond the Milan criteria, generally accepted limit for liver transplant treatment. The only way to improve survival in patients with HCC is to make diagnosis in an asymptomatic stage, through a surveillance program. This is possible because we know who are the patients at risk of developing HCC. Main risk factors for HCC include chronic infections for HBV and HCV, alcoholic liver disease (ALD) and MAFLD, with some geographic differences. In East Asia and Africa, hepatitis B is the first etiology being aflatoxin contamination a cofactor in some regions whereas in western countries, hepatitis C and alcoholic cirrhosis are the main causes of HCC. NASH and cryptogenic cirrhosis (probably "burnt-out" NASH) have historically ranked third in series from the United States [11] and Latin America[12]. However, changes are currently taking place and 2 recent studies agreed that NASH is the most rapidly growing indication for liver transplant among patients with HCC in United States[13,14]. According to the most recently published study, NASH now accounts for 18% of all HCC cases who are listed, meaning an 8-fold growth since 2002, and being the second most frequent cause, after hepatitis C[14].

Regarding which MAFLD patients to include in surveillance protocols for HCC, there are some discrepancies between the recommendations of the main Scientific Societies. Based on historical modeling studies performed in hypothetical patients with chronic hepatitis C, surveillance for HCC is assumed to be cost-effective when the annual incidence rate is equal to or greater than 1.5%. Therefore, all the recommendations agree that patients with MAFLD and cirrhosis should be included in that protocols; but they differ in respect to patients with advanced fibrosis (bridging fibrosis or F3 fibrosis). The last "Practice Guidance on Management of HCC" by the American Association for the Study of Liver Diseases states that "the risk of HCC is significantly lower in those with HCV or NAFLD without cirrhosis compared to those with cirrhosis, surveillance not being recommended for the former"[15]. The last Guidelines on "Management of hepatocellular carcinoma" by the European Association for the Study of the Liver affirms that "the role of surveillance for patients with NAFLD without cirrhosis is unclear (evidence low)"[10]; and in a table that lists "Categories of adult patients in whom surveillance is recommended", it states "Non-cirrhotic F3 patients, regardless of etiology may be considered for surveillance based on an individual risk assessment (evidence low; recommendation weak)[10]. On the contrary, the "American Gastroenterological Association Clinical Practice Update on Screening and Surveillance for HCC in Patients with Nonalcoholic Fatty Liver Disease", in its second Best Practice Advice, states that "patients with NAFLD with non-invasive markers showing evidence of advanced liver fibrosis or cirrhosis should be considered for HCC screening"[16].

It is worth mentioning that, unlike what happens with patients with hepatitis C or ALD, where HCC arises in cirrhotic liver in approximately 90% of cases; several case-control studies assessing characteristics of HCC in NAFLD patients have shown a significantly lower percentage of cirrhosis in NAFLD cases than in other etiology controls. The prevalence of cirrhosis among MAFLD cases with HCC has ranged from as low as 51%[17], 52.8%[18], 53.8%[19] to 77.2%[20]. Although fibrosis, cirrhosis and HCC appear to be the generic responses to any kind of chronic liver injury, the time-course and sequence of events appear to be even less predictable in MAFLD than other liver diseases. Nevertheless, in large population-based studies performed in non-selected NAFLD patients, the incidence rate of HCC is extremely low[21]. Although occasionally some case of HCC may appear in a patient with NAFLD and non-advanced fibrosis, it is generally considered that the risk is too low to justify the use of surveillance in all comers.

In this review, we will update the knowledge on epidemiological aspects of HCC in patients with MAFLD and analyze which are the factors that increase the risk between patients with and without cirrhosis. To address this, we will answer the following questions: What is the incidence of HCC in MAFLD, with and without cirrhosis? What are the clinical risk factors of HCC in MAFLD? What are the genetic phenotypes associated with HCC in MAFLD? Which are the differences between HCC diagnosed in patients with MAFLD compared to other etiologies? How can we "best screen" HCC in MAFLD at 2021?

It is important to recognize that, although the acronyms NAFLD and MAFLD refer to the same disease and will be used interchangeably in this review, all available information comes from studies previously conducted in patients with NAFLD.

INCIDENCE RATE OF HCC AND FACTORS THAT INCREASE THE RISK IN PATIENTS WITH MAFLD AND CIRRHOSIS

Several studies have compared the incidence rate of HCC between cohorts of patients with advanced

fibrosis/cirrhosis due to NAFLD or cryptogenic cirrhosis with HCV-related advanced fibrosis/cirrhosis cohort[22-27]. The Table 1 shows the main results of them.

One of the most representative studies within this group is that of Ascha and his colleagues at Cleveland Clinic[26]. Their primary objectives were to estimate the incidence of HCC between patients with NASH-cirrhosis and HCV-cirrhosis; and secondary, to identify risk factors for the occurrence of HCC. They reviewed data from 195 patients with NASH-cirrhosis and 315 patients with HCV-cirrhosis who had been referred for liver transplantation. The median age was significantly higher in patients with NASH than HCV (56.6 and 48.2 years, respectively, $P < 0.001$); but significantly fewer NASH patients were men compared with HCV patients (44.1% *vs* 76.5%, respectively; $P < 0.001$]. During a median follow-up of 3.2 years, HCC was diagnosed in 25 of 195 (12.8%) NASH-cirrhotic patients compared with 64/315 (20.3%) HCV-cirrhotic patients ($P = 0.03$). The annual cumulative incidence of HCC in patients with NASH cirrhosis was 2.6% compared with 4.0% for patients with HCV cirrhosis ($P = 0.09$)[26]. In multivariate analysis, they observed that older age [hazard ratio (HR), 1.08 (95%CI: 1.02-1.1); $P = 0.006$] and any alcohol consumption [HR, 3.8 (95%CI: 1.6-8.9); $P = 0.002$] were the only factors independently associated with development of HCC in the population with NASH-cirrhosis[26].

In other study, Sanyal *et al*[23] prospectively compared outcomes between 152 patients with NASH-related cirrhosis matched with 150 patients with HCV-cirrhosis[23]. Baseline characteristics of both groups were comparable in respect to liver function tests, Child-Pugh and MELD scores though patients with NASH had a higher frequency of metabolic syndrome features, such as diabetes or arterial hypertension. Over a 10-year follow-up, patients with NASH-cirrhosis had a significantly higher cardiac mortality than HCV cirrhotic patients ($P = 0.03$). By contrast, patients with HCV-cirrhosis had a significantly higher rate of general mortality ($P = 0.04$), development of ascites ($P < 0.006$) or progression to liver decompensation than patients with NASH-cirrhosis. In addition, patients with HCV had a significantly higher risk of developing HCC than NASH patients [17% (25/147) *vs* 6.7% (10/149), respectively, $P < 0.01$] (subtracting 3 patients from each group who had HCC at baseline). In Sanyal study, however, no HCC related risk factors were identified[23].

A large prospective, multicenter, international study compared the course of 247 patients with NAFLD and biopsy-proven advanced fibrosis or cirrhosis with that of 264 patients with hepatitis C and similar fibrosis stages (F3-F4)[27]. Patients with NAFLD were older (54.7 years *vs* 48.3 years, respectively, $P < 0.001$) with a higher percentage of females than HCV patients (60.3% *vs* 35.2%, respectively, $P < 0.001$). Mean follow-up were 85.6 and 74.9 mo in NAFLD group compared to HCV, respectively. After adjusting for baseline differences in age and gender, the cumulative incidence of liver-related complications was lower in the NAFLD than in the HCV cohort ($P = 0.03$), including incident HCC (6 *vs* 18 cases; $P = 0.03$). Among the 247 patients with NAFLD, 118 (47.8%) had F3 fibrosis and 129 (52.2%), Child A cirrhosis at the baseline. All cases of HCC in NAFLD group occurred among patients with cirrhosis (6 out of 129, 4.6%). In this study, no predictive factors for the development of HCC were identified[27].

A study from Japan retrospectively compared outcomes between 68 patients with NASH-cirrhosis and 69 matched HCV-cirrhosis patients[25]. The 5-year occurrence rate of HCC was 11.3% in the NASH group *vs* 30.5% in the HCV group. HCC incidence showed a slightly higher rate in the HCV group, but the difference was not significant ($P = 0.185$). An important finding of this study was that HCC was the leading cause of death in both groups (9 deaths in the NASH group and 19 in the HCV group)[25]. Moreover, in multivariate analysis, risk factors for the HCC occurrence were not identified.

Another study from France, retrospectively analyzed survival and cirrhosis complications in 27 overweight patients with cryptogenic cirrhosis and 391 patients with HCV-cirrhosis[22]. Patients with cryptogenic cirrhosis plus obesity were older than HCV cirrhotic patients (62.1 and 53.7 years, respectively; $P < 0.001$) but the sex ratio (male/female) was not significantly different between both groups. To avoid bias in the results based by the older age of cryptogenic cirrhosis plus obesity patients, a further analysis matched by age and low or no alcohol consumption compared outcomes between them and 85 HCV-cirrhotic patients[22]. The French study showed a slightly higher occurrence of HCC in the group with cryptogenic cirrhosis plus obesity than HCV cirrhosis group (30% *vs* 21%, respectively) though this tendency was not statistically significant[22].

In summary, this set of studies comparing outcomes between patients with NAFLD and hepatitis C and advanced fibrosis/cirrhosis mostly showed a slightly lower incidence of HCC in patients with NAFLD than in patients with HCV[23-27]. Only one of them[26] had the primary objective of investigating the incidence of HCC in NASH cirrhosis and found an annual incidence rate of 2.6%; while the others analyzed the appearance of cirrhosis complications in general. The yield of HCC incident cases in the individual studies was not as high and this may have prevented for identification of independent risk factors for HCC development at the multivariate analysis. Ascha *et al*[26] found that older age and any alcohol consumption were independent predictors of HCC occurrence[26]. To be noted, excessive alcohol intake is excluded (by former definition) in NAFLD patient groups but alcohol consumption may have played a role in HCV patient cohorts. This issue may not have been analyzed in detail in retrospective studies.

A large retrospective study by Kanwal *et al*[28] was conducted analyzing the Veterans Health Administration (VHA) database in United States, with the objective of estimating the risk of incident HCC among patients with MAFLD[28]. They compared 296.707 NAFLD patients with 296.707 matched

Table 1 Studies that evaluated hepatocellular carcinoma risk in a cohort with cirrhosis or advanced fibrosis due to nonalcoholic steatohepatitis or cryptogenic cirrhosis (presumptively nonalcoholic steatohepatitis-related) and in a comparison cohort with hepatitis C virus-related cirrhosis or advanced fibrosis

Ref.	*n*	Age (yr)	Male gender (%)	HCC incidence	*P* value
Ratziu *et al*[22]	27 CC-O	62.1 ± 10.6	(M/F) 1.7	8/27 (29.6%)	NS
	85 HCV	62.1 ± 10.6	(M/F) 1.7	18/85 (21%)	
Sanyal *et al*[23]	152 NASH	54.7 ± 11.6	39.7	10/149 (6.7%)	NS
	150 HCV	48.3 ± 11.3	64.8	25/147 (17%)	
Kojima *et al*[24]	24 CC	58.2 ± 10.6	NA	9/24 (37.5%)	*P* < 0.01
	48 HCV	58.7 ± 8.1	NA	36/48 (75%)	
Yatsuji *et al*[25]	68 NASH	62.7 ± 13.2	43	5-yr 11.3%	NS
	69 HCV	61.3 ± 5.8	43	5-yr 30.5%	
Ascha *et al*[26]	195 NASH	56.6	44.1	Annual cumulative 2.6%	*P* = 0.09
	315 HCV	48.2	76.5	Annual cumulative 4.0%	
Bhala *et al*[27]	247 NAFLD	54.7	39.5	6/247 (2.4%)	*P* = 0.03
	264 HCV	48.3	67.5	18/264 (6.8%)	

NAFLD: Nonalcoholic fatty liver disease; HCC: Hepatocellular carcinoma; NASH: Nonalcoholic steatohepatitis; HCV: Hepatitis C virus; CC-O: Overweight patients with cryptogenic cirrhosis; (M/F): Male/female ratio; NS: Non-significant; CC: Cryptogenic cirrhosis.

controls. Patients with NAFLD-cirrhosis had an annual incidence of HCC of 10.6/1000 persons-year (PYs). Among patients with cirrhosis, HCC incidence ranged from 1.6 to 23.7/1000 PYs, depending on other demographic characteristics like male gender, age older than 65 year and Hispanic race. The annual incidence *per* 1000 PYs (95%CI) was 11.05 (9.83–12.39) in men *vs* 1.62 (0.20–5.85) in women with cirrhosis; or 13.43 (10.82–16.49) in older than 65 years *vs* 9.74 (8.46–11.17) in younger than 65 years; or 23.76 (12.27–41.50) in Hispanics *vs* 11.94 (9.11–15.37) in Whites. The risk of HCC was the highest in older Hispanics with cirrhosis[28].

In a recently published paper, investigators from Mayo Clinic at Rochester, United States, assessed, as primary aim, the association of diabetes and HCC in patients with NASH and cirrhosis. Secondary aim was to analyze the association between other metabolic risk factors and HCC[29]. The retrospective cohort included 354 patients who did not have HCC at baseline. Mean age was 61.5 years and 41% were male. Diabetes was present in 253 (71%) patients at baseline. Follow-up duration was a median of 46 and 47 mo for diabetics and nondiabetics, respectively. HCC was diagnosed in 30 cases, 27 out of 253 patients with diabetes and 3 out of 101 patients without diabetes. The 5-year cumulative incidence rate of HCC was 7.8% (95%CI: 5.1-11.8) in the total population: 10.2% (95%CI: 6.6-15.5) for diabetics *vs* 1.7% (95%CI: 2.4-11.5) for nondiabetics[29]. In multivariable analysis, 3 factors were identified as independent predictors of HCC development: Older age, serum albumin levels, and diabetes[29]. In addition, authors revised the United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Network (OPTN) registry data to identify all adult patients who were registered on the waitlist for liver transplant in United States with diagnosis of NASH [or cryptogenic cirrhosis plus body mass index (BMI) ≥ 30] between 2003 and 2016. There were 6.630 patients with diagnosis of cirrhosis due to NAFLD, of whom 58% had diabetes. The 5-year cumulative incidence rate of HCC was 5.6% (95%CI: 4.9-6.3). Multivariate analysis showed that age, male sex, diabetes and low serum albumin level were independent risk factors for developing HCC[29].

Corey *et al*[30] performed a retrospective case-control study of patients with cirrhosis due to NAFLD followed-up in 5 academic centers from United States[30]. They evaluated 244 patients with NAFLD cirrhosis: 94 cases with HCC and 150 controls without HCC. Mean age was 59 years, male sex in 54.7%. On multivariate analysis, the strongest association with presence of HCC was male gender (OR = 4.3, 95%CI: 1.83-10.3, *P* = 0.001). Age was associated with HCC as well (OR = 1.082, 95%CI: 1.03-1.13, *P* = 0.001); and Hispanic ethnicity (contrary to what was described in the VHA study[28]) was associated with a decreased prevalence of HCC (OR = 0.3, 95%CI: 0.09-0.994, *P* = 0.048)[30].

CHARACTERISTICS OF PATIENTS WITH MAFLD WHO DEVELOP HCC

Several studies have shown that patients with HCC emerging in MAFLD have some significant

differences from patients with HCC arising in other chronic liver diseases. Firstly, patients with NAFLD plus HCC use to be older than other etiologies HCC[11,20,22,31]. Secondly, tumors tend to be larger as a result of a higher percentage of NAFLD patients being diagnosed outside of a surveillance program[19, 20,32]. As a consequence, some studies showed a shorter survival in patients with NAFLD and HCC compared to controls with other etiologies of cirrhosis and HCC[11,22]. However, other studies have found a similar[19,20] or longer[31] overall survival in NAFLD patients with HCC even though they had a more advanced stage. This could be due to the third and very important difference: A lower percentage of cirrhosis in MAFLD patients who develop HCC compared to their controls with hepatitis B or C or alcoholic liver disease and HCC[17-20,31,33]; giving them greater access to surgical resections of tumors. In fact, a recently published review evaluated the outcomes of patients with NAFLD and HCC who underwent surgical resection, finding that HCC occurs frequently in non-cirrhotic livers. Authors stated that all the papers showed a better overall survival after surgery in patients with NAFLD compared to other etiologies[34].

The Table 2 shows studies assessing presence of cirrhosis in patients with HCC due to NAFLD *vs* other etiologies.

In summary, the incidence of HCC in patients with MAFLD related cirrhosis show a rate that is above the accepted threshold as cost-effective to indicate surveillance protocols. Among the predictors of increased risk of developing HCC, several studies coincided in favor of the presence of male gender, older age and type 2 diabetes as independently significant risk factors. Also, we would like to point out the study by Ascha *et al*[26] (vide supra) where any degree of alcohol consumption (in patients who by the former definition do not have a significant intake) may increase the risk of HCC occurrence.

INCIDENCE RATE OF HCC AND FACTORS THAT INCREASE THE RISK IN PATIENTS WITH MAFLD WITHOUT CIRRHOSIS

In the previously mentioned retrospective study based on the VHA database (vide supra), analyzing more than 290.000 NAFLD patients and more than 290.000 matched controls, only 0.4% of patients had a diagnosis of cirrhosis at baseline and other 1.4% were confirmed as having cirrhosis later during the study. Mean follow-up was approximately 9 years in both groups. The annual HCC incidence rate was estimated in 0.21/1000 PYs (95%CI: 0.19-0.22) for NAFLD patients; significantly higher than that found in controls, 0.02/1000 PYs (95%CI: 0.02-0.03). NAFLD was associated with a 7.6-fold higher risk of HCC, after adjusting for race and metabolic syndrome features. Multivariate analysis showed that factors that significantly increase the risk of HCC among NAFLD patients are presence of diabetes [adj. HR 3.03 (95%CI: 2.52–3.64), $P < 0.0001$], age ≥ 65 years [adj. HR 1.83 (95%CI: 1.53–2.18), $P < 0.0001$] and Hispanic ethnicity [adj. HR 1.59 (95%CI: 1.14–2.20), $P = 0.005$][28].

Studies based on the general population that evaluate incidence rate of HCC in patients with NAFLD usually show a fairly low risk. In an elegant study from Taiwan, Lee *et al*[35] using Taiwan's National Health Insurance Research Database, evaluated the HCC incidence rate of NAFLD cohort comparing with general population. They recruited 18.080 patients with NAFLD, with a median follow-up of 6.32 years. The 10-year cumulative incidence of HCC was 2.73% (95%CI: 1.69–3.76%) in the total cohort[35]. Multivariate analysis verified that elevated alanine aminotransferase (ALT) was independently associated with an increased HCC risk [HR 6.80, (95%CI: 3.0-15.42), $P < 0.001$]. Another independent risk factor identified was age [HR 1.08 *per* year, (95%CI: 1.05–1.11)]; and statin use was independently associated with a reduction in HCC risk [HR 0.29, (95%CI: 0.12–0.68)]. By combining 2 independent risk factors, the risk of HCC can be better stratified: 10-year cumulative HCC incidence was highest in older (age > 55 years) patients with elevated ALT (12.41%, 95%CI: 5.99–18.83%), but lowest in younger patients without ALT elevation (0.36%, 95%CI: 0–1.08%)[35].

In another large study based on the general population, data were extracted from four European primary care databases (United Kingdom, Netherlands, Italy and Spain)[36]. Subjects who had a recorded diagnosis of NAFLD or NASH were analyzed up for incident cirrhosis and HCC diagnoses and each NAFLD patient was matched up to 100 controls by practice site, gender and age. Among 18.782.281 adults, 136.703 patients with coded NAFLD/NASH were identified. Hazard ratio for HCC in patients compared to controls was 3.51 (95%CI: 1.72–7.16). The strongest independent predictor of a diagnosis of HCC or cirrhosis was baseline presence of diabetes, which doubled the risk of developing these outcomes (HR 2.3, 95%CI: 1.9–2.78)[36].

The study by Adams and colleagues at Mayo Clinic, based on the general population using the Rochester Epidemiology Project, showed that patients with NAFLD have a lower survival than the control population[37]. They identified 420 patients diagnosed with NAFLD (mostly by imaging methods) and compared their overall survival and liver morbidity with the general Minnesota population of the same age and sex. In a mean follow-up of 7.6 years, 53 of 420 (12.6%) patients died. Standardized mortality ratio of NAFLD cohort was 1.34 (95%CI: 1.003–1.76; $P < 0.03$). Independent predictors of mortality were age, impaired fasting glucose and cirrhosis (HR, 3.1, 95%CI: 1.2–7.8)[37]. Only 21 (5%) patients were diagnosed with cirrhosis during this relatively short period of time, and 2 developed HCC. Even so, liver disease was the third leading cause of death among NAFLD patients as

Table 2 Studies that analyzed the prevalence of cirrhosis among patients with nonalcoholic fatty liver disease-related hepatocellular carcinoma and in controls with other etiologies-related hepatocellular carcinoma

Ref.	*n*	Prevalence of cirrhosis (in percentage)
Ertle *et al*[18]	36 NAFLD	52.8
	35 HCV	94.3 in HCV
	29 HBV	93.1 in HBV
	19 ALD	94.7 in ALD
Reddy *et al*[31]	52 NAFLD	73.1
	162 HCV/ALD	93.8
Dyson *et al*[20]	136 NAFLD	77.2
	178 ALD	100 in ALD
	65 HCV	96.9 in HCV
	29 HBV	82.7 in HBV
Mittal *et al*[33]	107 NAFLD	65.4
	1133 ALD	88.9 in ALD
	952 HCV	91.1 in HCV
	65 HBV	92.3 in HBV
Piscaglia *et al*[19]	145 NAFLD	53.8
	611 HCV	97.2 in HCV
Yasui *et al*[17]	87	51
	No control group	NA

NAFLD: Nonalcoholic fatty liver disease; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBV: Hepatitis B virus; ALD: Alcoholic liver disease; NA: Not available.

compared with the 13rd leading cause of death in control population[37].

Studies conducted in hospitals (clinic-based studies) presumably include "sicker" patients, in whom the risk of outcomes such as cirrhosis or HCC might be increased. However, in the follow-up of unselected NAFLD patients enrolled from an ultrasound (US) diagnosis, the incidence of HCC may continue to be extremely low. In a Japanese retrospective cohort study, 6.508 patients with NAFLD diagnosed by abdominal US were followed-up for 5.6 years. The cumulative rates of HCC were 0.02% at year 4, 0.19% at year 8, and 0.51% at year 12[38]. The annual rate of incident HCC was 0.043%. In this study, multivariate analysis identified 4 independent risk factors for developing HCC: Serum aspartate aminotransferase (AST) level $\geq$ 40 IU/L [HR 8.20; (95%CI: 2.56– 26.26) $P < 0.001$]; platelet count < 150 × 10^3/μL [HR 7.19; (95%CI: 2.26–23.26) $P = 0.001$]; age $\geq$ 60 years [HR 4.27; (95%CI: 1.30–14.01) $P = 0.017$] and diabetes [HR 3.21; (95%CI: 1.09–9.50) $P = 0.035$][38].

In another large retrospective study based on the VHA database, Kanwal *et al*[39] evaluated the independent and joint effects of different metabolic traits (diabetes, hypertension, dyslipidemia and obesity) on the risk of developing cirrhosis and HCC[39]. The cohort consisted of 271.906 patients with NAFLD who did not have cirrhosis or HCC at baseline, mean age was 55.5 ± 12.8 years, 94.3% were men, 28.7% had diabetes, 70.3% had arterial hypertension, mean BMI was 31.6 ± 5.6. During a mean follow-up of 9.3 years, 22.794 patients (8.4%) progressed to cirrhosis whereas HCC was diagnosed in 253 patients. Diabetes was the only factor independently associated with the risk of HCC by the multivariate analysis, the risk of HCC was nearly 2.8-fold higher than those without diabetes (adjusted HR = 2.77, 95%CI: 2.03-3.77). Obesity and dyslipidemia were associated with 31% increase in HCC risk. However, these associations, like hypertension, did not reach statistical significance[39].

In another clinic-based Japanese, retrospective study, 1600 older than 60 years NAFLD patients (diagnosed by US) and 1600 older than 60 years matched HCV patients were enrolled with the aim of investigating the cumulative incidence of malignant diseases, including HCC[40]. At the baseline, there were several significant differences between both groups. Metabolic parameters such as triglycerides, total cholesterol, fasting plasma glucose were more elevated in NAFLD patients, but AST, ALT, alpha-fetoprotein (AFP) were more elevated and platelet count more decreased in hepatitis C patients[40]. No data was described on the liver fibrosis stages of the patients. Mean observation period was 8.2 years in both groups. Cumulative development rate of malignant diseases at the 10th year was 13.9% in the

NAFLD group and 28.2% in the HCV group by Kaplan–Meier method (risk ratio 2.27; *P* < 0.001). However, the incidence rate of HCC was clearly pronounced in the HCV cohort, where 267 HCC cases were diagnosed (20.86/1000 PY) compared to 10 HCC cases diagnosed in the NAFLD group (0.78/1000 PY) (*P* < 0.001)[40]. In the NAFLD group, multivariate analysis showed that independent predictors of malignancies were age of ≥ 70 years (HR 2.10; 95%CI: 1.38–3.17; *P* < 0.001), current smoking (HR 1.64; 95%CI: 1.18–2.27; *P* = 0.003), and elevated glucose level (HR 1.32; 95%CI: 1.08–1.61; *P* = 0.007)[40].

Dam-Larsen *et al*[41,42] published 2 papers examining the long-term prognosis of 2 cohorts of patients, one with nonalcoholic fatty liver (NAFL) and the other, with alcoholic fatty liver (AFL)[41,42]. In the first study, they evaluated the risk of development of cirrhosis and death in 215 patients (109 with NAFL and 106 with AFL) who had underwent a liver biopsy. All the patients had biopsy-proven single steatosis, without NASH. During a median follow up time of 16.7 years in the NAFL and 9.2 years in the AFL group, only one NAFL patient developed cirrhosis compared with 22 patients in the alcoholic group[41]. Survival estimates in NAFL group were not different from the Danish population. In the last study, the aim was to conduct an extended, clinical follow-up in both NAFL and AFL patients, to analyze for potential risk factors for survival and development of cirrhosis, and to describe the causes of death[42]. This second analysis enrolled 170 patients with NAFL and 247 patients with AFL whose liver biopsies had been taken between the years 1976 and 1987. All surviving patients were contacted in 2003 and invited to attend a clinical follow up visit. Median follow-up times in the whole cohort were 20.7 years and 12.8 years in the NAFL and AFL groups, respectively. Two (1.2%) patients with NAFL and 54 (22%) with AFL, respectively, were diagnosed as having cirrhosis during follow-up. Forty-eight NAFL patients died during the study period and one of them died from cirrhosis. Within the AFL group, 188 patients died, 32 of them (17%) from cirrhosis. Regarding HCC as a cause of death, there was no cases in NAFLD group and one in AFL group[42].

Many studies have been published attempting to assess the risk of HCC or other liver complications in patients with non-cirrhotic NAFLD, but they have many limitations and weaknesses. Most of them were retrospective and heterogeneous in terms of the inclusion criteria; did not have data on liver fibrosis stages; or they had too short a follow-up to assess hard outcomes such as HCC or complications of cirrhosis. In addition, most of them had relatively few cases of HCC diagnosed and multivariate analysis trying to identify risk factors were powerless. Therefore, it is difficult to draw conclusions or make recommendations on in whom to indicate surveillance for HCC in patients with MAFLD, especially when there is no information on liver fibrosis. Table 3 summarizes some studies that analyzed the incidence rate of HCC in patients with NAFLD without cirrhosis and which were the independent risk factors found in the multivariate analysis.

At the same time, it is important to note that large epidemiological studies carried out in the general population have shown a significant association between the presence of diabetes, or obesity and even metabolic syndrome and PLC.

DIABETES MELLITUS, OBESITY, METABOLIC SYNDROME AND RISK OF PLC

The association of diabetes and PLC has been established for many years. A Swedish population-based cohort study analyzed the risk of developing PLC and biliary tract cancers among 153.852 patients with diabetes, identifying incident cases of cancer during follow-up through the Swedish Cancer Registry [43]. The incidence of PLC was increased fourfold (standardized incidence ratio = 4.1; 95%CI: 3.8-4.5). Even after excluding diabetic patients with concomitant conditions that predispose to HCC, such as alcoholism, cirrhosis, and hepatitis, it was observed an excess risk of approximately threefold[43].

El–Serag *et al*[44] identified all patients with a hospital discharge diagnosed of diabetes between 1985 and 1990 using the records of VHA and assigned randomly 3 controls for every patient, excluding those with concomitant liver disease[44]. The study cohort included 173.643 patients with diabetes and 650.620 controls without diabetes, followed through 2000 for the occurrence of NAFLD related cirrhosis or HCC. Diabetes was associated with 2-fold increase for HCC (HR 2.16, 95%CI: 1.86-2.52, *P* < 0.0001), independently of alcoholic liver disease, viral hepatitis, or demographic features[44].

A multicenter Italian hospital-based study also found that body mass index ≥ 30 kg/m^2 and diabetes mellitus (OR 3.7, 95%CI: 1.7–8.4) were associated to HCC risk[45]; and these associations persisted among subjects without HBV and/or HCV infection[45].

After many cohort studies suggested a strong association between type 2 diabetes mellitus and HCC, a systematic review and meta-analysis was performed, including 25 cohort studies[46]. Diabetes mellitus was associated with an increased incidence of HCC [summary relative risks (SRR) = 2.01, 95%CI: 1.61–2.51]. Increased incidence of HCC in patients with diabetes was independent of geographic location, alcohol consumption, history of cirrhosis, or infections with HBV or HCV[46].

Multiple studies have suggested that metformin, a first-line diabetes medication, may reduce the incidence of HCC and other cancers. Although the mechanism is not well understood, this was initially shown in animal models of HCC and then assessed in many human studies. A recent meta-analysis of 19 studies involving 550.882 diabetic patients concluded that metformin use reduced the liver cancer incidence by 48% (OR 0.52; 95%CI: 0.40–0.68) compared with nonusers[47]. The association remained

Table 3 Incidence rate of hepatocellular carcinoma and independent risk factors among patients with non-alcoholic fatty liver disease/metabolic-dysfunction associated fatty liver disease without cirrhosis

Ref.	Population studied	*n*	Mean follow-up (yr)	Incidence rate of HCC	Independent risk factors
Kanwal *et al* [28]	VHA database (United States)	295.623	9.0 ± 2.2	0.08/1000 person-years	Male gender; Age > 65 yr; hispanics
Lee *et al*[35]	General population-based study (Taiwan of China)	18.081	Median 6.32	10-yr cumulative incidence 2.73% (95%CI: 1.69–3.76)	Age > 55 yr; elevated ALT
Alexander *et al* [36]	General population-based study (Europe)	136.703	Median 3.3	0.3/1000 person-years	Diabetes
Kawamura *et al* [38]	Clinic-based study (Japan)	6.508	Median 5.6	Annual incidence 0.043%	AST ≥ 40 IU/L; platelet count < 150×10^3 /μL; age > 60 yr; diabetes
Arase *et al*[40]	Clinic-based study (Japan)	1.600	8.2	0.78/1000 person-years	Age > 70 yr; smoking; elevated glucose level
Kanwal *et al* [39]	VHA database (United States)	271.906	9.3 ± 2.7	253 cases[1]	Diabetes

[1]Incidence rate of hepatocellular carcinoma was not calculated.

HCC: Hepatocellular carcinoma; VHA: Veterans Health Administration; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

after adjusting for hepatitis B/C virus infection, cirrhosis, obesity, behavioral factors, and time-related bias. Sensitivity analysis showed that the beneficial effect of metformin was observed in 10 cohort studies and in 9 case-control studies but not when 2 randomized controlled trials were considered separately (they were probably underpowered or with short period of follow-up)[47]. To avoid time-related biases, a propensity score-matched retrospective cohort was constructed enrolling 84.434 veterans newly prescribed metformin or a sulfonylurea as monotherapy (42.217 new metformin users and 42.217 matched-new sulfonylurea users. Metformin treatment was associated with a reduction in liver cancer [adj. HR 0.44, (95%CI: 0.31-0.64)] compared to sulfonylurea therapy[48]. In subgroup analysis, metformin compared to sulfonylurea was also associated with lower liver cancer incidence in both patients with and without baseline cirrhosis and when the cohort was stratified by statin use at baseline[48].

At the same time, many cohort studies have shown association between overweight, obesity and risk of liver cancer. Already in 2007, a meta-analysis including 10 studies with 6.042 cases, concluded that, compared with normal weight individuals, the SRR of liver cancer was 1.89 (95%CI: 1.51–2.36) for those with obesity[49]. Subsequently, more case-control studies were published and the meta-analyzes were updated. One of them, published in 2012, included 26 prospective studies and more than 25.000 PLC cases. Obesity was associated with an increased risk of PLC (SRRs 1.83, 95%CI: 1.59–2.11)[50], and this association was even further in obese males than obese females (*P* = 0.027). Subgroup analyses revealed that positive associations were independent of geographic locations, alcohol consumption, history of diabetes or infections with HBV and/or HCV[50]. Therefore, body of evidence suggests that obesity increases the risk of HCC, that is approximately twice that of normal weight individuals. However, it is still uncertain whether there is a gender difference in the association between obesity and PLC. A new meta-analysis was conducted to quantitatively and precisely evaluate the gender difference in that association[51]. The results showed increased relative risks (RR) of HCC incidence for obese men than women (RR 2.04, 95%CI: 1.70–2.44 *vs* RR 1.56, 95%CI: 1.37–1.78, respectively, *P* = 0.02)[51]. Furthermore, the RR's of HCC incidence for men and women were compared between non-Asian and Asian countries. The RR's of HCC incidence were significantly higher in obese men than obese women in non-Asian studies (RR 2.31, 95%CI: 1.85–2.91, *vs* RR 1.56 (95%CI: 1.31–1.86, respectively, *P* = 0.01) but not in Asian countries[51].

More importantly, there is a linear relationship between increasing BMI and death from various types of cancer, including PLC[52]. A prospective investigation was conducted in United States in a large cohort of men and women with the aim to determine the relations between BMI and the risk of death from cancer at specific sites. More than 900.000 adults (free of cancer at baseline) were enrolled in 1982. During 16 years of follow-up there were 57.145 deaths from cancer. As compared with men of normal weight, men with a BMI ≥ 35.0 had significantly elevated RRs of death from cancer, which ranged from 1.23 (95%CI: 1.11 to 1.36) for death from any cancer to 4.52 (95%CI: 2.94 to 6.94) for death from liver cancer. There was a significant positive linear trend in death rates with increasing BMI for several types of cancers (esophageal, stomach, colorectal, pancreatic, gallbladder cancers, *etc.*) but the one with the highest risk was the PLC, which was increased by 4-5 times in men with BMI > 35[52].

Furthermore, metabolic syndrome (as defined by the United States National Cholesterol Education Program Adult Treatment Panel III criteria) has also been shown to be a significant risk factor for

development of HCC in the general population. Cases of HCC (*n* = 3.649) were identified in the Surveillance, Epidemiology, and End Results (SEER)-Medicare database, in United States. Control group was composed by 195.953 persons residing in the same regions. By adjusted multiple logistic regression analyses, metabolic syndrome was significantly associated with increased risk of HCC (OR 2.13; 95%CI: 1.96-2.31, *P* < 0.0001)[53].

GENETIC VARIANTS ASSOCIATED WITH HCC IN MAFLD

Recent advances in the field of Genetics allow obtaining comprehensive data on the genetic alterations associated with MAFLD-related HCC. Genome wide association studies (GWAS) look for links between single nucleotide polymorphism (SNP) and disease phenotype. Differential gene expression results from gene mutations in regulatory elements or epigenetic changes, which plays an important role in susceptibility to the development of HCC. Of over 100 Loci examined in GWAS and candidate gene studies, genetic variations in 5 genes have emerged as reproducibly and robustly predisposing individuals to development of MAFLD (*PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7* and *HSD17B13*)[54]. While unexplained variance remains despite these discoveries, indicating that future GWAS may reveal additional associations[54].

The patatin-like phospholipase domain-containing protein 3 (PNPLA3) genetic mutation on rs738409 c.444C>G SNP is a well-known risk factor for hepatic steatosis, disease severity, fibrosis stage and progression to NAFLD-related HCC[55-58]. This variant was most common in Hispanics, who are more susceptible to MAFLD[59]. Singal *et al*[60] performed a systematic review and meta-analysis of 24 studies with 9.915 patients and found that PNPLA3 was associated with an increased risk of HCC in patients with cirrhosis (OR 1.40, 95%CI: 1.12–1.75)[60]. Upon subgroup analysis, PNPLA3 was found to be an independent risk factor for HCC in patients with NAFLD or alcoholic liver disease-related cirrhosis (OR 1.67, 95%CI: 1.27-2.21), but not among other etiologies[60]. Liu *et al*[57] in a case-control study of 100 NAFLD related HCC and 275 controls with histologically characterized NAFLD, reported that bearing the PNPLA3 rs738409 c.444C>G was associated with GG homozygotes exhibiting a 5-fold increased risk of HCC in patients with NAFLD and when compared with United Kingdom general population the risk-effect was even more pronounced[57]. This association persisted following multivariate adjustment for age, gender, diabetes, BMI and presence of cirrhosis[57]. Interestingly, its effects to promote the full spectrum of NAFLD are modulated by interactions with environmental factors[61] and other gene variants[58,62].

A rs58542926 c.449C>T SNP in transmembrane-6-superfamily member 2 (TM6SF2) gene is associated with increased liver fat content, NASH and fibrosis progression[58,63-65]. Noteworthy, the TM6SF2 rs58542926 c.449C>T variant is associated with lower levels of serum cholesterol, LDL-cholesterol and triglycerides, and is protective against cardiovascular disease[65]. To evaluate the association between NAFLD-related HCC risk and TM6SF2 rs58542926 c.449C>T variant, Liu and coworkers reported that the TM6SF2 variant confers increased predisposition to NAFLD-related HCC (OR 1.922, 95%CI: 1.31–2.81)[64]. However, this association was not significant when risk factors including gender, age, diabetes and cirrhosis were considered by multivariate analysis[64].

A SNP in the glucokinase regulator (GCKR), rs1260326 and rs780094 variants are associated with increased susceptibility to NAFLD and fibrosis progression[66-69]. However, only GCKR rs1260326 variant predispose to NASH-related HCC (OR 1.84 95%CI: 1.23-2.75)[69]. Both GCKR variants interact with PNPLA3 rs738409 c.444C>G in elevating susceptibility to NASH in people with both risk alleles[66,68].

A SNP rs641738 g.54173068 C>T variant of the gene encoding membrane bound O-acyltransferase domain-containing 7 (MBOAT7) has been linked with an increased risk of hepatic steatosis, inflammation and fibrosis[70,71]. To ascertain the NAFLD-related HCC risk with MBOAT7 rs641738 variant, Donati and coworkers evaluated an Italian cohort of 765 NAFLD patients where MBOAT7 rs641738 variant was associated with NAFLD-HCC independently of clinical factors or fibrosis stage (OR 2.18, 95%CI: 1.30–3.63)[72].

The rs72613567 T>TA in the hydroxysteroid 17-β dehydrogenase 13 gene (HSD17B13) have recently been linked with a reduced risk of chronic liver disease[73]. The HSD17B13 rs72613567 variant in NAFLD-cohort patients is associated with decreased steatohepatitis and severe fibrosis[73,74]. Associations between the HSD17B13 rs72613567 variant and reduced odds of HCC in a variety of etiologies including NAFD and ALD have been reported[75,76].

Since MAFLD is a complex disease, therefore, it is logical that combining genetic variants into a risk score will improve prognostic accuracy over a singular genetic variant. Based on this concept, Donati and coworkers observed a significant association between the number of risk alleles variants (PNPLA3, TM6SF2 and MBOAT7) and HCC (OR *per* allele 1.56, 95%CI: 1.31–1.86; OR complete positive alleles 9.25, 95%CI: 3.83–22.8) that was not affected after adjusted for clinical factors and fibrosis stage[72]. In this cohort, HCC risk was 9% in the population with 0–1 risk allele, 19% in the population with 2–3 risk alleles, and 31% in the population with ≥ 4 risk alleles[72]. In the same work, Donati *et al*[72] developed a combined clinical and polygenic risk score (PRS) to predict HCC, the model had a 0.96 ± 0.4 area

under the receiving operating characteristic curve (AUROC) for detecting HCC cases, with optimal cutoff of 96% sensitivity and 89% specificity for HCC risk in the Italian NAFLD cohort. Recently, Gellert-Kristensen and coworkers demonstrated that a PRS, combining the 3 genetic variants in *PNPLA3*, *TM6SF2* and *HSD17B13*, was associated with risk of cirrhosis and HCC in fatty liver disease (both NAFLD and alcohol-related) from Denmark and United Kingdom general population[77]. The score ranged from 0 to 6 depending on the number of risk alleles, a score of ≥ 5 was associated with a 12-fold increased risk of cirrhosis and a 29-fold increased risk of HCC[77]. Bianco and colleagues, evaluated a PRS to improve HCC risk stratification in NAFLD (*n* = 1699) and general population cohort (United Kingdom BioBank), combining PNPLA3-TM6SF2-GCKR-MBOAT7 (PRS) and then adjusted for HSD17B13 (PRS-5). In the NAFLD cohort, PRS were associated with an approximately 12-fold increased odds of severe fibrosis and an approximately 9-fold increased odds of HCC (OR 9.2, 95%CI: 5.2–16.3 for PRS; and OR 9.1, 95%CI: 5.2–16.0 PRS-5)[78]. The association was independent of age, gender, diabetes and BMI but not of severe fibrosis. In the NAFLD cohort, the AUROC for HCC was 0.64 for PRS and 0.65 for PRS-5, the best single cut-off for PRS with 43% sensitivity and 80% specificity and for PRS-5 with 43% sensitivity and 79% specificity[78]. These promising polygenic risk prediction scores adjusted for conventional risk factors may, in the future, have the potential to guide care of patients with MAFLD. It is likely that genetic risk variants will need to be combined with other variables, such as clinical parameters, to improve score performance[72].

SCREENING TESTS FOR HCC SURVEILLANCE IN PATIENTS WITH MAFLD

In addition to deciding which patients with MAFLD should be involved in surveillance protocols for HCC, it would be necessary to address which are the best screening tests. For many years now, Scientific Societies have recommended the use of hepatic US with or without serum AFP measurement every 6 mo, based on its cost-effectiveness, acceptability for patients, easy accessibility and HCC doubling time. However, the sensitivity of this strategy to detect tumors eligible for curative treatment is not ideal. The ultimate goal of HCC surveillance is to increase patient survival, and for this, early stage tumors (within Milan criteria) must be diagnosed. A recent meta-analysis showed that sensitivities of liver US alone or with AFP measurement to detect early-stage HCC were 45% and 63%, respectively [79]. Furthermore, inadequate liver ultrasound quality may be more common in overweight or obese patients.

A retrospective cohort study was conducted to determine factors associated with inadequate US quality in HCC surveillance. Among 941 US examinations performed in cirrhotic patients, 191 (20.3%) studies were considered as inadequate for excluding HCC[80]. By multivariate analysis, inadequate quality was associated with male gender, BMI category, Child–Pugh B or C, and alcoholic or NASH related cirrhosis. In NASH-cirrhosis, the risk of having an inadequate US quality increased almost 3 times (OR 2.87, 95%CI: 1.71–4.80); and hepatic US was inadequate in over one-third of patients with BMI > 35[80].

The adequacy of ultrasound in assessing the cirrhotic liver to exclude nodular lesions depends not only on the patient factors but also of the operator. However, there are no regulations worldwide on the expertise that a radiologist must have to perform US examinations in the heterogeneous cirrhotic liver for HCC surveillance. The LI-RADS group (endorsed by the American College of Radiology) has proposed a US visualization score[81], where A category means "No or minimal limitations"; B, "Moderate limitations". In B category, the concept is "limitations may obscure small masses". Examples are moderate beam attenuation or some portions of the liver were not visualized. Finally, score C means "Severe limitations", the concept is "Limitations significantly lower sensitivity for focal liver lesions" and examples are majority (> 50%) of liver or diaphragm were not visualized[81]. When the US visualization score is B or particularly when is C, an additional imaging method should be indicated as screening test (computed tomography or magnetic resonance imaging). Furthermore, patients at higher risk for HCC (*e.g.*, NASH cirrhosis plus diabetes), even with adequate US, should perhaps be screened with a more sensitive test.

A prospective Korean study by Kim *et al*[82] was performed to compare the HCC detection rate of hepatic US and liver-specific contrast enhanced magnetic resonance imaging (MRI) in patients with cirrhosis who were at high risk for HCC[82]. They enrolled 407 patients with cirrhosis and an estimated annual risk of HCC greater than 5% who underwent 1 to 3 biannual screening examinations with paired US and liver-specific contrast-enhanced MRI. HCC were diagnosed in 43 patients. They found that the HCC detection rate of MRI was 86.0% (37/43), significantly higher than the 27.9% (12/43) of US (*P* < 0.001) and 74.4% of tumors were in a very-early stage (a single nodule < 2 cm), with 67.4% of patients receiving curative treatments[82].

MRI is not used routinely in surveillance protocols for HCC because it would not be cost-effective in patients with low or intermediate risk. MRI is less affordable, more expensive and much more time consuming than US. However, in patients with very high HCC risk or in whom US is suboptimal, MRI could become the primary screening test. To minimize the costs and scanning time, protocols of abbreviated MRI (AMRI) are being tested[83,84] showing high percentages of sensitivity and specificity

in the preliminary results. AMRI protocols will play a role in the future in surveillance for HCC in patients at high risk or in whom the quality of US is inadequate.

CONCLUSION

The increasing prevalence of overweight, obesity, insulin resistance and type 2 diabetes have made MAFLD the most common chronic liver disease and a real challenge for physicians and health systems worldwide. Patients with NASH and advanced fibrosis or cirrhosis are the most prone to developing complications of cirrhosis including HCC. The diagnosis of NASH can only be confirmed by liver biopsy. However, given the huge number of patients with MAFLD and the invasiveness of the method, most patients will not undergo a liver biopsy. Nevertheless, the stage of liver fibrosis (and not the condition NASH/no NASH) is the main driver of liver-related morbidity and mortality. Non-invasive tests (NITs) to assess liver fibrosis stage, especially the non-proprietary ones such as FIB4[85] and the NAFLD fibrosis score[86], and different types of elastography[87] are being used with increasing frequency in the management of patients with NAFLD. The most appropriate algorithm to determine liver fibrosis in patients with MAFLD is beyond the scope of this review. Briefly, when a combination of 2 NITs (*e.g.*, FIB4 plus elastography or FIB4 plus NAFLD fibrosis score) yields a result below the low cut-off value, this has a high negative predictive value to rule out advanced fibrosis/cirrhosis in patients with NAFLD. It is assumed that the patient will not present liver-related morbidity in the short time and it is not necessary to include this patient in a surveillance protocol for HCC.

On the other end, patients with an unequivocal diagnosis of cirrhosis should be involved in surveillance for HCC. In most of them, conventional screening tests will be used, liver US plus serum AFP measurement every 6 mo; following the recommended recall procedures when any of the tests yield a positive result[10,15]. As mentioned, in a percentage of patients with NASH cirrhosis and/or with obesity, the quality of the US will not be adequate to confidently rule out nodular liver lesions. In addition, it may happen that patients with NAFLD and cirrhosis have factors that significantly increase the risk of HCC. In these 2 situations, the primary screening test could be MRI or an AMRI protocol. According to our review, factors that have been repeatedly found to increase the risk of HCC in cirrhosis due to MAFLD are male gender, older age, the presence of diabetes and, in some studies, decreased levels of serum albumin and any degree of alcohol intake. Table 4 suggests different risk categories for HCC in patients with NAFLD/MAFLD.

The risk of HCC occurrence is much more difficult to stratify in patients with MAFLD who do not have a diagnosis of cirrhosis. As mentioned, the "AGA Clinical Practice Update on Screening and Surveillance for Hepatocellular Carcinoma in Patients with NAFLD" states that patients with NITs showing evidence of advanced liver fibrosis or cirrhosis should be considered for HCC screening[16]. That expert panel recommends to combine at least 2 NITs and to better stratify risk and maximize specificity, they propose using higher than usual cut-off values for noninvasive detection of cirrhosis: 16.1 kPa for transient elastography; and 5 kPa for magnetic resonance elastography[16].

In the majority of patients with NAFLD (approximately 55%-60%) the NITs exclude advanced fibrosis and in a minority of them (approximately 10%-15%), they confirm severity of liver damage. However, there are a large number of patients in whom NITs show indeterminate results, which do not allow to rule out or confirm advanced fibrosis/cirrhosis. This gray area is where it is most challenging to stratify the risk of HCC or other complications of cirrhosis. According to findings of population and clinic-based studies in patients with diagnosis of NAFLD by US that we have revised, elevated AST levels[38] and decreased platelet counts[38] (both could be surrogates of severe fibrosis) and also elevated ALT levels [35] increase independently the risk of HCC. Furthermore, in many of these studies older age[28,35,38, 40] and diabetes[28,36,38,39] were shown to be independent risk factors for HCC occurrence as well. In addition, multiple large, population-based studies have shown that individuals with diabetes[43-47], obesity[45,49-52], or metabolic syndrome[53] have a 2-3 times higher risk of HCC occurrence than their controls in the population analyzed. All of these epidemiological, cross-sectional studies demonstrate the association between diabetes, obesity, metabolic syndrome and HCC, without being able to analyze the causal relationship and the mechanisms involved. It is assumed that the link between these metabolic traits and the appearance of HCC is through the hepatic manifestation of metabolic syndrome, NASH, advanced fibrosis and cirrhosis. Finally, the study of genetic alterations that predispose to MAFLD, advanced fibrosis and increased risk of HCC will be an important tool in the near future, when PRSs become easily available. Only 2 factors have been associated with a significant decrease in the risk of HCC occurrence: Metformin use in diabetic patients[47,48] and statin use in NAFLD patients[35].

Figure 1 shows the main factors that can increase the risk of HCC in patients with MAFLD. It is worth to emphasize that the most influential risk is the presence of cirrhosis. In patients with cirrhosis, there is no doubt that they should be included in surveillance for HCC, with conventional tests or occasionally, with MRI.

In patients without cirrhosis but in whom NITs suggest the presence of advanced fibrosis, it seems reasonable to indicate surveillance for HCC with conventional screening tests, US plus AFP, twice a
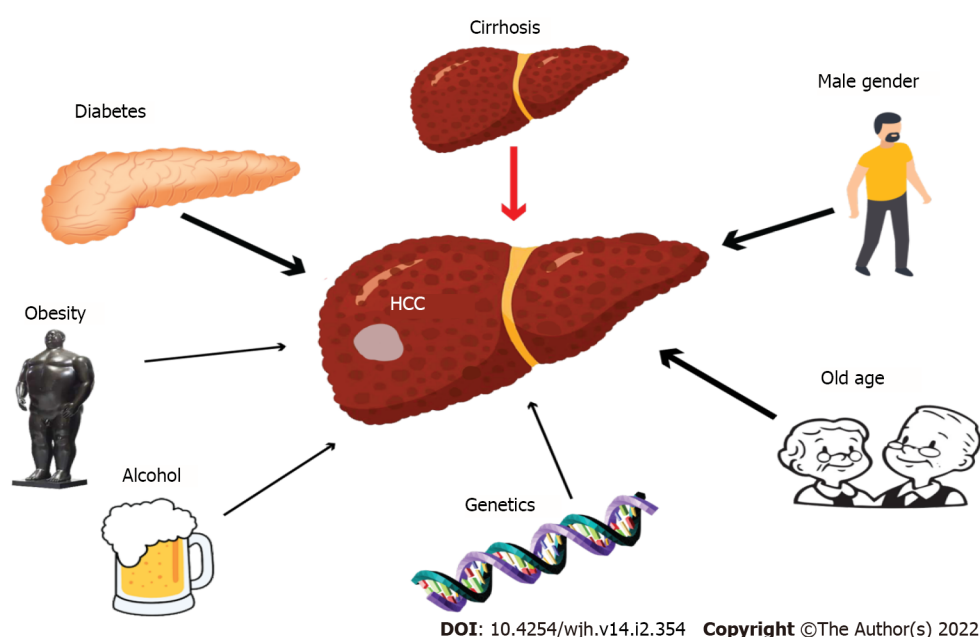
Table 4 Different risk categories for hepatocellular carcinoma in patients with non-alcoholic fatty liver disease/metabolic-dysfunction associated fatty liver disease[1]

Risk for HCC	Patients	
Very high	Cirrhosis plus	Male gender
		Older age
		Diabetes
		Low serum albumin
High	Cirrhosis	
	F3 fibrosis plus	Elevated AST[2]
		Low platelets[2]
		Older age
		Diabetes
Low	F3 fibrosis	
Very low	Mild or no liver fibrosis	

[1]Study of polygenic risk score will be important for better stratification of hepatocellular carcinoma risk when clinically available.
[2]Probably understaged liver fibrosis.
HCC: Hepatocellular carcinoma.

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Figure 1 Factors that can increase the risk of hepatocellular carcinoma in patients with non-alcoholic fatty liver disease/metabolic-dysfunction associated fatty liver disease. Presence of cirrhosis is the factor that has the greatest influence on the incidence of hepatocellular carcinoma. HCC: Hepatocellular carcinoma.

year. In patients in whom NITs show intermediate results, there is, to date, no recommendation that can be made based on scientific evidence. The treating physician should consider the presence of additional risk factors that were described in this review and decide accordingly. As an example, a male patient, older than 65 years and with diabetes or elevated AST has a higher risk of HCC than a female patient, 45 years old, without diabetes or with normal AST. Further prospective, longitudinal, cooperative studies have to be carried out in this group of patients to better understand the risk of HCC and which factors may modify its incidence. This will allow better risk stratification, optimize surveillance and improve tests adequacy.

ACKNOWLEDGEMENTS

FOOTNOTES

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